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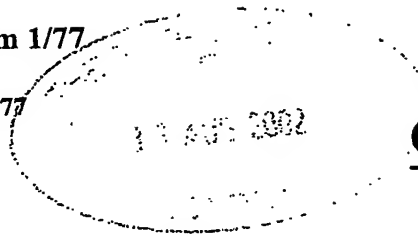

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Glaxo Group Limited
Glaxo Wellcome House, Berkeley Avenue,
Greenford, Middlesex UB6 0NN, Great Britain

Patents ADP number *(if you know it)* 00473587003

If the applicant is a corporate body, give the country/state of its incorporation — United Kingdom

4. Title of the invention — Vaccine

5. Name of your agent *(if you have one)*

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R J Easeman

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Vaccine

The present invention relates to isolated immunogens and their use in the treatment of COPD, and asthma. In particular the invention relates to raising an immune response against the cytokine IL-13 by vaccination of a mammal with immunogens comprising the autologous amino acid sequence of IL-13, and foreign T-helper epitopes either inserted in, or attached to the autologous IL-13 sequence or present in carrier polypeptides. Also provided by the present invention are DNA vaccines that comprise a polynucleotide sequence that encodes the immunogens of the present invention. The invention further relates to pharmaceutical compositions comprising such immunogens and their use in medicine and to methods for their production.

COPD is an umbrella term to describe diseases of the respiratory tract, which shows similar symptoms to asthma and is treated with the same drugs. COPD is characterised by a chronic, progressive and largely irreversible airflow obstruction. The contribution of the individual to the course of the disease is unknown, but smoking cigarettes is thought to cause 90% of the cases. Symptoms include coughing, chronic bronchitis, breathlessness and respiratory injections. Ultimately the disease will lead to severe disability and death.

Asthma is a chronic lung disease, caused by inflammation of the lower airways and is characterised by recurrent breathing problems. Airways of patients are sensitive and swollen or inflamed to some degree all the time, even when there are no symptoms. Inflammation results in narrowing of the airways and reduces the flow of air in and out of the lungs, making breathing difficult and leading to wheezing, chest tightness and coughing. Asthma is triggered by super-sensitivity towards allergens (e.g. dust mites, pollens, moulds), irritants (e.g. smoke, fumes, strong odours), respiratory infections, exercise and dry weather. The triggers irritate the airways and the lining of the airways swell to become even more inflamed, mucus then clogs up the airways and the muscles around the airways tighten up until breathing becomes difficult and stressful and asthma symptoms appear.

As a result of the various problems associated with the production, administration and tolerance of monoclonal antibodies there is an increased focus on methods of instructing the patient's own immune system to generate endogenous antibodies of the appropriate specificity by means of vaccination. However, mammals do not generally have high-titre antibodies against self-proteins present in serum, as the immune system contains homeostatic mechanisms to prevent their formation. The importance of these "tolerance" mechanisms is illustrated by diseases like myasthenia gravis, in which auto-antibodies directed to the nicotinic acetylcholine receptor of skeletal muscle cause weakness and fatigue (Drachman, 1994, *N Engl J Med* 330:1797-1810).

A number of techniques have been designed with the aim of breaking "tolerance" to self antigen. One technique involves chemically cross-linking the self-protein (or peptides derived from it) to a highly immunogenic carrier protein, such as keyhole limpet haemocyanin ("Antibodies: A laboratory manual" Harlow, E and Lane D. 1988. Cold Spring Harbor Press).

A variant on the carrier protein technique involves the construction of a gene encoding a fusion protein comprising both carrier protein (for example hepatitis B core protein) and self-protein (The core antigen of hepatitis B virus as a carrier for immunogenic peptides", Biological Chemistry. 380(3):277-83, 1999). The fusion gene may be administered directly as part of a nucleic acid vaccine. Alternatively, it may be expressed in a suitable host cell *in vitro*, the gene product purified and then delivered as a conventional vaccine, with or without an adjuvant.

Another approach has been described by Dalum and colleagues wherein a single class II MHC-restricted epitope is inserted into the target molecule. They demonstrated the use of this method to induce antibodies to ubiquitin (Dalum et al, 1996, *J Immunol* 157:4796-4804; Dalum et al, 1997, *Mol Immunol* 34:1113-1120) and the cytokine TNF (Dalum et al, 1999, *Nature Biotech* 17:666-669). As a result, all T cell help must arise either from this single epitope or from junctional sequences. Such an approach is also described in EP 0 752 886 B1, WO 95/05849, and WO 00/65058.

WO 00/65058 describes a method of downregulating the function of the cytokine IL-5, and its use in the treatment of asthma. In this study, the IL-5 sequence was modified by a number of techniques to render it immunogenic, amongst which there is described an IL-5 immunogen supplemented with foreign T-cell epitopes, whilst maintaining the IL-5 B cell epitopes.

A number of recent papers have defined the role for the Th2 cytokine IL-13 in driving pathology in the ovalbumin model of allergenic asthma (Wills-Karp et al, 1998, *Science* 282:2258-2261; Grunig et al, 1998, *Science* 282:2261-2263). In this work, mice previously sensitised to ovalbumin were injected with a soluble IL-13 receptor which binds and neutralises IL-13. Airway hyper-responsiveness to acetylcholine challenge was reduced in the treated group. Histological analysis revealed that treated mice had reversed the goblet-cell metaplasia seen in controls. In complementary experiments, lung IL-13 levels were raised by over-expression in a transgenic mouse or by installation of protein into the trachea in wild-type mice. In both settings, airway hyper-responsiveness, eosinophil invasion and increased mucus production were seen (Zhu et al, 1999, *J.Clin.Invest.* 103:779-788).

The sequence of the mature form of human IL-13 is provided in SEQ ID No. 1.

G P V P P S T A L R E L I E E L V N I T Q N Q K A
P L C N G S M V W S I N L T A G M Y C A A L E S L
I N V S G C S A I E K T Q R M L G G F C P H K V S
A G Q F S S L H V R D T K I E V A Q F V K D L L L
H L K K L F R E G R F N *

The sequence of the mature form of murine IL-13 is provided in SEQ ID No. 2.

G P V P R S V S L P L T L K E L I E E L S N I T Q D
Q T P L C N G S M V W S V D L A A G G F C V A L D
S L T N I S N C N A I Y R T Q R I L H G L C N R K

A P T T V S S L P D T K I E V A H F I T K L L S Y
T K Q L F R H G P F *

The present invention provides pharmaceutical compositions comprising modified "self" IL-13 immunogens, wherein the IL-13 immunogen is modified to include foreign T-cell helper epitopes. The pharmaceutical composition is preferably for use in human therapy, and in this composition the IL-13 sequence is a human sequence or other sequence that is capable of generating an immune response against human IL-13; and the T-cell helper epitopes are foreign with respect to human self-proteins. Preferably the T-helper epitopes are also foreign with respect to other IL-13 sequences from other species. However, animal pharmaceutical products are not excluded, for example canine or other veterinary species pharmaceutical products can be made in an analogous fashion to that described for human vaccines above.

Preferably the T-cell helper epitopes are small and are added to the IL-13 sequence by an addition or substitution event within or at the terminal ends of the IL-13 sequence by synthetic, recombinant or molecular biological means. Alternatively the T-cell helper epitopes may be added via chemical coupling of the IL-13 polypeptide to a carrier protein comprising the T-cell helper epitopes. In the context of the present invention the entire IL-13 sequences may be used, or functional equivalent fragments thereof. The IL-13 sequences, or functionally equivalent fragments thereof, may also be associated with the T-cell helper epitopes in a fusion protein, wherein the two are recombinantly manufactured together, for example a Hepatitis B core protein incorporating IL-13 sequences.

Also provided by the present invention is the use of these modified IL-13 immunogens in the manufacture of a medicament for the treatment of COPD, or for the treatment of asthma. Also provided is a method of treatment COPD or asthma comprising the administration to an individual in need thereof of a pharmaceutical composition or vaccine as described herein.

Preferably the pharmaceutical composition is a vaccine that raises an immune response against IL-13. The immune response raised is preferably an antibody response, most preferably an IL-13 neutralising antibody response.

The invention also provides:

- an expression vector which comprises a polynucleotide of the invention and which is capable of expressing a polypeptide of the invention;
- a host cell comprising an expression vector of the invention;

a method of producing a polypeptide of the invention which method comprises maintaining a host cell of the invention under conditions suitable for obtaining expression of the polypeptide and isolating the said polypeptide:

a vaccine composition comprising a polypeptide or polynucleotide of the invention and a pharmaceutically acceptable carrier.

In the aspects of the present invention where small T-cell helper epitopes are used, a "foreign T-cell helper epitope" or "T-cell epitope" is a peptide which is able to bind to an MHC II molecule and stimulates T-cells in an animal species. Preferred foreign T-cell epitopes are promiscuous epitopes, *ie.* epitopes that bind multiple different MHC class II molecules in an animal species or population (Panina-Bordignon et al, *Eur.J.Immunol.* 1989, 19:2237-2242; Reece et al, *J.Immunol.* 1993, 151:6175-6184; WO 95/07707).

In order for the immunogens of the present invention to be sufficiently clinically effective, it may be necessary to include several foreign T-cell epitopes. Promiscuous epitopes according to the invention can be naturally occurring human T-cell epitopes such as those from tetanus toxoid (e.g. the P2 and P30 epitopes, diphtheria toxoid, influenza virus haemagluttinin (HA), and P.falciparum CS antigen. The most preferred T-cell epitopes for use in the present invention are P2 and P30 from tetanus toxoid

A number of promiscuous T-cell epitopes have been described in the literature, including: WO 98/23635; Southwood et al., 1998, J. Immunol., 160: 3363-3373; Sinigaglia et al., 1988, Nature, 336: 778-780; Rammensee et al., 1995, Immunogenetics, 41: 4, 178-228; Chicz et al.,

1993, J. Exp. Med., 178:27-47; Hammer et al., 1993, Cell 74:197-203; and Falk et al., 1994, Immunogenetics, 39: 230-242. The promiscuous T-cell epitope can also be an artificial sequence such as "PADRE" (WO 95/07707).

The heterologous T-cell epitope is preferably selected from the group of epitopes that will bind to a number of individuals expressing more than one MHC II molecules in humans. For example, epitopes that are specifically contemplated are P2 and P30 epitopes from tetanus toxoid, Panina – Bordignon Eur. J. Immunol 19 (12), 2237 (1989). In a preferred embodiment the heterologous T-cell epitope is P2 or P30 from Tetanus toxin.

The P2 epitope has the sequence QYIKANSKFIGITE and corresponds to amino acids 830-843 of the Tetanus toxin.

The P30 epitope (residues 947-967 of Tetanus Toxin) has the sequence FNNFTVSFWLRVPKVSASHLE. The FNNFTV sequence may optionally be deleted. Other universal T epitopes can be derived from the circumsporozoite protein from Plasmodium falciparum – in particular the region 378-398 having the sequence DIEKKIAKMEKASSVFNVVNS (Alexander J, (1994) Immunity 1 (9), p 751-761). Another epitope is derived from Measles virus fusion protein at residue 288-302 having the sequence LSEIKGVIVHRLEGV (Partidos CD, 1990, J. Gen. Virol 71(9) 2099-2105). Yet another epitope is derived from hepatitis B virus surface antigen, in particular amino acids, having the sequence FFLLTRILTIPQSLD.

Another set of epitopes is derived from diphteria toxin. Four of these peptides (amino acids 271-290, 321-340, 331-350, 351-370) map within the T domain of fragment B of the toxin, and the remaining 2 map in the R domain (411-430, 431-450):

PVFAGANYAAWAVNVAQVI

VHHNTEEIVAQSIALSSLMV

QSIALSSLMVAQAIPLVGEL

VDIGFAAYNFVESII NLFQV

QGESGHDIKITAENTPLPIA

GVLLPTIPGKLDVNKSKTHI

(Raju R., Navaneetham D., Okita D., Diethelm-Okita B., McCormick D., Conti-Fine B. M. (1995) Eur. J. Immunol. 25: 3207-14.)

Most preferably the foreign T-cell helper epitopes are "foreign" in that they are not tolerated by the host immune system, and also in that they are not sequences that are derived or selected from any IL-13 sequence from another species (non-vaccinee).

The methods of treatment of the present invention provide a method of treatment of asthma comprising one or more of the following clinical effects:

1. A reduction in airway hyper-responsiveness (AHR)
2. A reduction in mucus hyper-secretion and goblet cell metaplasia
3. A reduction in sub-epithelial fibrosis of the airways
4. A reduction in eosinophil levels
5. A reduction in the requirement for the use of inhaled corticosteroids (ICS) would also be a feature of successfull treatment using an IL13 autovaccine.

In the aspect of the present invention where native self IL-13 is coupled to a T-helper epitope bearing immunogenic carrier, the conjugation can be carried out in a manner well known in the art. Thus, for example, for direct covalent coupling it is possible to utilise a carbodiimide, glutaraldehyde or (N-[γ-maleimidobutyryloxy] succinimide ester, utilising common commercially available heterobifunctional linkers such as CDAP and SPDP (using manufacturers instructions). After the coupling reaction, the immunogen can easily be isolated and purified by means of a dialysis method, a gel filtration method, a fractionation method etc.

The types of carriers used in the immunogens of the present invention will be readily known to the man skilled in the art. A non-exhaustive list of carriers which may be used in the present invention include: Keyhole limpet Haemocyanin (KLH), serum albumins such as bovine serum albumin (BSA), inactivated bacterial toxins such as tetanus or diptheria toxins (TT and DT), or recombinant fragments thereof (for example, Domain 1 of Fragment C of TT, or the translocation domain of DT), or the purified protein derivative of tuberculin (PPD). Alternatively the IL-13 may be directly conjugated to liposome carriers, which may additionally comprise immunogens capable of providing T-cell help. Preferably the ratio of

IL-13 to carrier molecules is in the order of 1:1 to 20:1, and preferably each carrier should carry between 3-15 IL-13 molecules.

In an embodiment of the invention a preferred carrier is Protein D from *Haemophilus influenzae* (EP 0 594 610 B1). Protein D is an IgD–binding protein from *Haemophilus influenzae* and has been patented by Forsgren (WO 91/18926, granted EP 0 594 610 B1). In some circumstances, for example in recombinant immunogen expression systems it may be desirable to use fragments of protein D, for example Protein D 1/3rd (comprising the N-terminal 100-110 amino acids of protein D (GB 9717953.5)).

Another preferred method of presenting the IL-13, or immunogenic fragments thereof, is in the context of a recombinant fusion molecule. For example, EP 0 421 635 B describes the use of chimaeric hepadnavirus core antigen particles to present foreign peptide sequences in a virus-like particle. As such, immunogens of the present invention may comprise IL-13 presented in chimaeric particles consisting of hepatitis B core antigen. Additionally, the recombinant fusion proteins may comprise IL-13 and a carrier protein, such as NS1 of the influenza virus. For any recombinantly expressed protein which forms part of the present invention, the nucleic acid which encodes said immunogen also forms an aspect of the present invention.

The following provides preferred specific immunogens.

SEQ ID No 3 is a human IL-13 with P30 inserted into the protein (substituted for the looped region between alpha helices C and D of human IL13) this is an example of a human version of an IL-13 autovaccine.

G P V P P S T A L R E L I E E L V N I T Q
N Q K A P L C N G S M V W S I N L T A G M
Y C A A L E S L I N V S G C S A K T Q G T
R M L G G F C P H K K S A G Q F S S L H V
V P K V S A S H L E D T K I E V A Q F V K
D L L L H L K K L F R E G R F N

SEQ ID NO. 89 is a Human IL-13 immunogen with N-terminal P30

F N N F T V S F W L R V P K V S A S H L E G P V P P S T A L R E L I E E L V N I
T Q N Q K A P L C N G S M V W S I N L T A G M Y C A A L E S L I N V S G C S A
I E K T Q R M L G G F C P H K K S A G Q F S S L H V R D T K I E V A Q F V K D
L L L H L K K L F R E G R F N

SEQ ID No. 4 is a murine IL-13 with p30 inserted into the protein (substituted for the looped region between alpha helices C and D of mouse IL13) this is an example of a mouse version of an IL13 autovaccine. The first 20 amino acids correspond to the mammalian signal peptide for secretion, this signal sequence does not remain in the mature secreted protein. The p30 region is highlighted in italics.

G P V P R S V S L P L T L K E L I E E L S N I T Q D Q T P L C N G S
M V W S V D L A A G G F C V A L D S L T N I S N C N A I Y R T Q
R I L H G L C N R K F N N F T V S F W L R V P K V S A S H L E D T
K I E V A H F I T K L L S Y T K Q L F R H G P F

SEQ ID NO. 5 is a murine IL13 with p30 at the N-terminus. This is an example of a mouse version of an IL13 autovaccine. The p30 region is highlighted in italics and is positioned at the N-terminus of the mature mouse IL13 protein sequence. The first 20 amino acids correspond to the mammalian signal peptide for secretion, this signal sequence does not remain in the mature secreted protein

F N N F T V S F W L R V P K V S A S H L E G P V P R S V S L P L T L
K E L I E E L S N I T Q D Q T P L C N G S M V W S V D L A A G G F
C V A L D S L T N I S N C N A I Y R T Q R I L H G L C N R K A P T
T V S S L P D T K I E V A H F I T K L L S Y T K Q L F R H G P F

In an alternative embodiment of the present invention the immunogens comprise foreign – helper epitopes and have a chimaeric IL-13 sequence. In this sense, in the case of a human IL-13 vaccine, the IL-13 immunogen will be based on an orthologous IL-13 sequence (such as the murine IL-13 sequence) wherein the murine B-cell epitopes (surface exposed regions) are substituted for the equivalent human sequences. In this embodiment the murine "backbone" will provide foreign T-cell epitopes, in addition to the supplemental promiscuous T-cell epitopes (such as P2 or P30) which are added either at the termini or within the chimaera sequence.

Such a construct is provided in SEQ ID No. 6. This is an example of a mouse version of this form of the vaccine, where there is "human backbone" sequence grafted to murine B-cell surface exposed epitopes, with P30 added at the N-terminus.

F N N F T V S F W L R V P K V S A S H L E G P V P R S V S L P V T L
K E L I E E L T N I T Q D Q T P L C N G S M V W S V D L A A G G
F C V A L D S L T N I S N C N A I F R T Q R I L H A L C N R K A P
T T V S S L P D T K I E V A H F I T K L L T Y T K N L F R R G P F

In certain aspects of the present invention immunogenic fragments of the native IL-13 sequence may be used, for example in the presentation of immunogenic peptides in Hepatitis B core particles or in the context of chimaeric immunogens described above. In these contexts immunogenic fragments of the human IL-13 sequences preferably contain the B-cell epitopes in the human IL-13 sequence, and preferably at least one or more of the following short sequences:

GPVPPSTA
ITQNQKAPLCNGSMVWSINLTAGM
INVSGCS
FCPHKVSAGQFSSLHVRDT
LHLKKLFREGRFN

Throughout this specification and the appended claims, unless the context requires otherwise, the words "comprise" and "include" or variations such as "comprising", "comprises", "including", "includes" etc., are to be construed inclusively, that is, use of these words will imply the possible inclusion of integers or elements not specifically recited.

As described herein, the present invention relates isolated polypeptides and isolated polynucleotides. In the context of this invention the term "isolated" is intended to convey that the polypeptide or polynucleotide is not in its native state, insofar as it has been purified at least to some extent or has been synthetically produced, for example by recombinant methods, or mechanical synthesis. The term "isolated" therefore includes the possibility of the polypeptides or polynucleotides being in combination with other biological or non-biological material, such as cells, suspensions of cells or cell fragments, proteins, peptides, expression vectors, organic or inorganic solvents, or other materials where appropriate, but excludes the situation where the polynucleotide is in a state as found in nature.

The successful design of a polypeptide according to the present invention can be verified for example by administering the resulting polypeptide in a self-context in an appropriate vaccination regime, and observing that antibodies capable of binding the protein are induced. This binding may be assessed through use of ELISA techniques employing recombinant or purified native protein, or through bioassays examining the effect of the protein on a sensitive cell or tissue. A particularly favoured assessment is to observe a phenomenon causally related to activity of the protein in the intact host, and to determine whether the presence of antibodies induced by the methods of the invention modulate that phenomenon. Thus a protein of the present invention will be able to raise antibodies to the native antigen in the species from which the native protein is derived.

The polypeptide of the invention may be further modified by mutation, for example substitution, insertion or deletion of amino-acids in order to add desirable properties (such as the addition of a sequence tag that facilitates purification or increase immunogenicity) or remove undesirable properties (such as an unwanted agonistic activity at a receptor) or trans-membrane domains. In particular the present invention specifically contemplates fusion partners that ease purification such as poly histidine tags or GST expression partners that enhance expression.

A "conservative substitution" is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydropathic nature of the polypeptide to be substantially unchanged.

For example, certain amino acids may be substituted for other amino acids in a protein structure without appreciable loss of interactive binding capacity with structures such as, for example, antigen-binding regions of antibodies or binding sites on substrate molecules. Since it is the interactive capacity and nature of a protein that defines that protein's biological functional activity, certain amino acid sequence substitutions can be made in a protein sequence, and, of course, its underlying DNA coding sequence, and nevertheless obtain a protein with like properties. It is thus contemplated that various changes may be made in the

peptide sequences of the disclosed compositions, or corresponding DNA sequences which encode said peptides without appreciable loss of their biological utility or activity.

In making such changes, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a protein is generally understood in the art (Kyte and Doolittle, 1982, incorporated herein by reference). It is accepted that the relative hydropathic character of the amino acid contributes to the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like. Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics (Kyte and Doolittle, 1982). These values are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (–0.4); threonine (–0.7); serine (–0.8); tryptophan (–0.9); tyrosine (–1.3); proline (–1.6); histidine (–3.2); glutamate (–3.5); glutamine (–3.5); aspartate (–3.5); asparagine (–3.5); lysine (–3.9); and arginine (–4.5).

It is known in the art that certain amino acids may be substituted by other amino acids having a similar hydropathic index or score and still result in a protein with similar biological activity, i.e. still obtain a biological functionally equivalent protein. In making such changes, the substitution of amino acids whose hydropathic indices are within ±2 is preferred, those within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred. It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. U. S. Patent 4,554,101 (specifically incorporated herein by reference in its entirety), states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein.

As detailed in U. S. Patent 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0 ± 1); glutamate (+3.0 ± 1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (–0.4); proline (–0.5 ± 1); alanine (–0.5); histidine (–0.5); cysteine (–1.0); methionine (–1.3); valine (–1.5);

leucine (–1.8); isoleucine (–1.8); tyrosine (–2.3); phenylalanine (–2.5); tryptophan (–3.4). It is understood that an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent, and in particular, an immunologically equivalent protein. In such changes, the substitution of amino acids whose hydrophilicity values are within ±2 is preferred, those within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred.

As outlined above, amino acid substitutions are generally therefore based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions that take various of the foregoing characteristics into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine. These are preferred conservative substitutions.

Amino acid substitutions may further be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine and valine; glycine and alanine; asparagine and glutamine; and serine, threonine, phenylalanine and tyrosine. Other groups of amino acids that may represent conservative changes include: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his.

The polypeptide of the invention may be encoded by polynucleotides of the invention. A person skilled in the art will readily be able to determine the sequence of the polynucleotide which encodes the polypeptide by applying the genetic code. Once the required nucleic acid sequence has been determined, the polynucleotide with the desired sequence can be produced as described in the examples. A skilled person will readily be able to adapt any parameters necessary, such as primers and PCR conditions. It will also be understood by a

person skilled in the art that, due to the degeneracy of the genetic code, there is potentially more than one polynucleotide which encodes a polypeptide of the invention. The polynucleotides of the present invention may also comprise a region which encodes a secretion signal peptide.

The polynucleotide of the invention is typically RNA, for example mRNA, or DNA, for example genomic DNA, cDNA or synthetic DNA. Preferably the polynucleotide is DNA. Particularly preferably it is cDNA.

The present invention further provides an expression vector, which is a nucleic acid construct, comprising the polynucleotide of the invention. Additionally, the nucleic acid construct will comprise appropriate initiators, promoters, enhancers and other elements, such as for example, polyadenylation signals, which may be necessary, and which are positioned in the correct orientation, in order to allow for protein expression within a mammalian cell.

The promoter may be a eukaryotic promoter for example a CD68 promoter, Gal1, Gal10, or NMT1 promoter, a prokaryotic promoter for example Tac, Trc, or Lac, or a viral promoter, for example the cytomegalovirus promoter, the SV40 promoter, the polyhedrin promoter, the P10 promoter, or the respiratory syncytial virus LTR promoter. Preferably the promoter is a viral promoter. Particularly preferred is when the promoter is the cytomegalovirus immediate early promoter, optionally comprising exon 1 from the HCMV IE gene.

The transcriptional regulatory elements may comprise enhancers, for example the hepatitis B surface antigen 3'untranslated region, the CMV enhancer; introns, for example the CD68 intron, or the CMV intron A, or regulatory regions, for example the CMV 5' untranslated region.

The polynucleotide is preferably operably linked to the promoter on the nucleic acid construct such that when the construct is inserted into a mammalian cell, the polynucleotide is expressed to produce a encoded polypeptide.

The nucleic acid construct backbone may be RNA or DNA, for example plasmid DNA, viral DNA, bacterial DNA, bacterial artificial chromosome DNA, yeast artificial chromosome

DNA, synthetic DNA It is also possible for the nucleic acid construct to be artificial nucleic acid, for example phosphorothioate RNA or DNA. Preferably the construct is DNA. Particularly preferred is when it is plasmid DNA.

The present invention further provides a host cell comprising an expression vector of the invention. Such cells include transient, or preferably stable higher eukaryotic cell lines, such as mammalian cells or insect cells, using for example a baculovirus expression system, lower eukaryotic cells, such as yeast or prokaryotic cells such as bacterial cells. Particular examples of cells which may be modified by insertion of vectors encoding for a polypeptide according to the invention include mammalian HEK293T, CHO, HeLa, NS0 and COS cells. Preferably the cell line selected will be one which is not only stable, but also allows for mature glycosylation of a polypeptide. Expression may be achieved in transformed oocytes. A polypeptide of the invention may be expressed in cells of a transgenic non-human animal, preferably a mouse or expressed into the milk of larger mammals, such as goats, sheep and cows. A transgenic non-human animal expressing a polypeptide of the invention is included within the scope of the invention. A polypeptide of the invention may also be expressed in *Xenopus laevis* oocytes.

The present invention also includes pharmaceutical or vaccine compositions, which comprise a therapeutically effective amount of nucleic acid construct or polypeptide of the invention, optionally in combination with a pharmaceutically acceptable carrier, preferably in combination with a pharmaceutically acceptable excipient such as phosphate buffered saline (PBS), saline, dextrose, water, glycerol, ethanol, liposomes or combinations thereof. The vaccine composition may alternatively comprise a therapeutically effective amount of a nucleic acid construct of the invention, formulated onto metal beads, preferably gold beads. The vaccine composition of the invention may also comprise an adjuvant, such as, for example, in an embodiment, imiquimod, tucaresol or aluminium salts.

Preferably the adjuvant is administered at the same time as the immunogens of the present invention, and in preferred embodiments are formulated together. Such adjuvant agents contemplated by the invention include, but this list is by no means exhaustive and does not

preclude other agents: synthetic imidazoquinolines such as imiquimod [S-26308, R-837], (Harrison, et al. 'Reduction of recurrent HSV disease using imiquimod alone or combined with a glycoprotein vaccine', Vaccine 19: 1820-1826, (2001)); and resiquimod [S-28463, R-848] (Vasilakos, et al. ' Adjuvant activites of immune response modifier R-848: Comparison with CpG ODN', Cellular immunology 204: 64-74 (2000).), Schiff bases of carbonyls and amines that are constitutively expressed on antigen presenting cell and T-cell surfaces, such as tucaresol (Rhodes, J. et al. ' Therapeutic potentiation of the immune system by costimulatory Schiff-base-forming drugs', Nature 377: 71-75 (1995)), cytokine, chemokine and co-stimulatory molecules, Th1 inducers such as interferon gamma, IL-2, IL-12, IL-15 and IL-18, Th2 inducers such as IL-4, IL-5, IL-6, IL-10 and IL-13 and other chemokine and co-stimulatory genes such as MCP-1, MIP-1 alpha, MIP-1 beta, RANTES, TCA-3, CD80, CD86 and CD40L, other immunostimulatory targeting ligands such as CTLA-4 and L-selectin, apoptosis stimulating proteins and peptides such as Fas, (49), synthetic lipid based adjuvants, such as vaxfectin, (Reyes et al., 'Vaxfectin enhances antigen specific antibody titres and maintains Th1 type immune responses to plasmid DNA immunization', Vaccine 19: 3778-3786) squalene, alpha- tocopherol, polysorbate 80, DOPC and cholesterol, endotoxin, [LPS], Beutler, B., 'Endotoxin, 'Toll-like receptor 4, and the afferent limb of innate immunity', Current Opinion in Microbiology 3: 23-30 (2000)) ; CpG oligo- and di-nucleotides, Sato, Y. et al., 'Immunostimulatory DNA sequences necessary for effective intradermal gene immunization', Science 273 (5273): 352-354 (1996). Hemmi, H. et al., 'A Toll-like receptor recognizes bacterial DNA', Nature 408: 740-745, (2000) and other potential ligands that trigger Toll receptors to produce Th1-inducing cytokines, such as synthetic Mycobacterial lipoproteins, Mycobacterial protein p19, peptidoglycan, teichoic acid and lipid A.

Certain preferred adjuvants for eliciting a predominantly Th1-type response include, for example, a Lipid A derivative such as monophosphoryl lipid A, or preferably 3-de-O-acylated monophosphoryl lipid A. MPL® adjuvants are available from Corixa Corporation (Seattle, WA; *see*, for example, US Patent Nos. 4,436,727; 4,877,611; 4,866,034 and 4,912,094). CpG-containing oligonucleotides (in which the CpG dinucleotide is unmethylated) also induce a predominantly Th1 response. Such oligonucleotides are well

known and are described, for example, in WO 96/02555, WO 99/33488 and U.S. Patent Nos. 6,008,200 and 5,856,462. Immunostimulatory DNA sequences are also described, for example, by Sato et al., *Science 273*:352, 1996. Another preferred adjuvant comprises a saponin, such as Quil A, or derivatives thereof, including QS21 and QS7 (Aquila Biopharmaceuticals Inc., Framingham, MA); Escin; Digitonin; or *Gypsophila* or *Chenopodium quinoa* saponins.

For the treatment of IL-13 mediated disease it is preferred that the adjuvant is a preferable inducer of a TH-1 response. In particular, the adjuvant comprises an immunostimulatory CpG oligonucleotide, such as disclosed in (WO96102555). Typical immunostimulatory oligonucleotides will be between 8-100 bases in length and comprises the general formula X_1 $CpGX_2$ where X_1 and X_2 are nucleotide bases, and the C and G are unmethylated.

The preferred oligonucleotides for use in vaccines of the present invention preferably contain two or more dinucleotide CpG motifs preferably separated by at least three, more preferably at least six or more nucleotides. The oligonucleotides of the present invention are typically deoxynucleotides. In a preferred embodiment the internucleotide in the oligonucleotide is phosphorodithioate, or more preferably a phosphorothioate bond, although phosphodiester and other internucleotide bonds are within the scope of the invention including oligonucleotides with mixed internucleotide linkages. e.g. mixed phosphorothioate/phophodiesters. Other internucleotide bonds which stabilise the oligonucleotide may be used. Methods for producing phosphorothioate oligonucleotides or phosphorodithioate are described in US5,666,153, US5,278,302 and WO95/26204.

Examples of preferred oligonucleotides have the following sequences. The sequences preferably contain phosphorothioate modified internucleotide linkages.

OLIGO 1: TCC ATG ACG TTC CTG ACG TT (CpG 1826)
OLIGO 2: TCT CCC AGC GTG CGC CAT (CpG 1758)
OLIGO 3: ACC GAT GAC GTC GCC GGT GAC GGC ACC ACG
OLIGO 4: TCG TCG TTT TGT CGT TTT GTC GTT (CpG 2006)

OLIGO 5: TCC ATG ACG TTC CTG ATG CT (CpG 1668)

Alternative CpG oligonucleotides may comprise the preferred sequences above in that they have inconsequential deletions or additions thereto.

The CpG oligonucleotides utilised in the present invention may be synthesized by any method known in the art (eg EP 468520). Conveniently, such oligonucleotides may be synthesized utilising an automated synthesizer. An adjuvant formulation for use in mice and containing CpG oligonucleotide can be purchased from Qiagen under the trade name "ImmunEasy". Preferably the adjuvant is one of the CpG's defines as OLIGO's 1, 2, 3, 4 or 5 adsorbed to aluminium hydroxide at an approximate 1:1 ratio weight/weight. OLIGO 4 is preferred for use in humans.

Preferably the CpG is in combination with a saponin, such as QS21, as described in WO 00/62800 and WO 00/09159 the contents of both of which is encorporated herein by reference.

The compositions of the present invention may be used for both prophylaxis and therapy. The present invention provides a polypeptide or a polynucleotide according to the invention for use in medicine. The invention further provides the use of a polypeptide or a polynucleotide of the invention in the manufacture of a medicament for the treatment of allergies, respiratory ailments such as asthma and COPD, helminth-infection related disorders, fibrosis or cirrhosis of the liver.

The present invention also provides a method of vaccinating which comprises administering an effective amount of a vaccine composition of the invention to a patient and provoking an immune response to the vaccine composition.

The present invention also provides vaccine compositions as described herein for use in vaccination of a mammal against IL-13 mediated disorders such as allergies, respiratory ailments, helminth-infection related disorders, fibrosis and cirrhosis of the liver. A vaccine composition capable of directing a neutralising response to IL-13 would therefore constitute a

useful therapeutic for the treatment of asthma, particularly allergic asthma, in humans. It would also have application in the treatment of certain helminth infection-related disorders (Brombacher, 2000 *Bioessays* 22:646-656) and diseases where IL-13 production is implicated in fibrosis (Chiaramonte et al, 1999, *J Clin Inv* 104:777-785), such as chronic obstructive pulmonary disease (COPD) and cirrhosis of the liver.

The present invention also provides methods of treating or preventing IL-13 mediated disease, any symptoms or diseases associated therewith, comprising administering an effective amount of a protein, a polynucleotide, a vector or a pharmaceutical composition according to the invention. Administration of a pharmaceutical composition may take the form of one or more individual doses, for example in a "prime-boost" therapeutic vaccination regime. In certain cases the "prime" vaccination may be via particle mediated DNA delivery of a polynucleotide according to the present invention, preferably incorporated into a plasmid-derived vector and the "boost" by administration of a recombinant viral vector comprising the same polynucleotide sequence, or boosting with the protein in adjuvant. Conversly the priming may be with the viral vector or with a protein formulation typically a protein formulated in adjuvant and the boost with a DNA vaccine of the present invention.

The present invention provides methods of generating an anti self IL-13 antibody response in a host by the administration of vaccines of the present invention.

The vaccine compositions of the invention may be administered in a variety of manners for example via the mucosal, such as oral and nasal; pulmonary, intramuscular, subcutaneous or intradermal routes. Where the antigen is to be administered as a protein based vaccine, the vaccine will typically be formulated with an adjuvant and may be lyophilised and resuspended in water for injection prior to use. Such compositions may be administered to an individual as an injectable composition, for example as a sterile aqueous dispersion, preferably isotonic. Typically such compositions will be administered intra muscularly, but other routes of administration are possible.

One technique for intradermally administration involves particle bombardment (which is also known as 'gene gun' technology and is described in US Patent No. 5371015). Proteins may be formulated with sugars to form small particles or DNA encoding the antigen may be coated on to inert particles (such as gold beads) and are accelerated at speeds sufficient to enable them to penetrate a surface of a recipient (e.g. skin), for example by means of discharge under high pressure from a projecting device. (Particles coated with nucleic acid vaccine constructs of the invention and protein sugar particles are within the scope of the present invention, as are devices loaded with such particles.) Other methods of administering the nucleic acid constructs or compositions containing said constructs directly to a recipient include ultrasound, electrical stimulation, electroporation and microseeding which is described in US-5,697,901.

A nucleic acid construct of the present invention may also be administered by means of specialised delivery vectors useful in gene therapy. Gene therapy approaches are discussed for example by Verme *et al*, Nature 1997, 389:239-242. Both viral and non-viral systems can be used. Viral based systems include retroviral, lentiviral, adenoviral, adeno-associated viral, herpes viral and vaccinia-viral based systems. Non-viral based systems include direct administration of nucleic acids and liposome-based systems. For example, the vectors may be encapsulated by liposomes or within polylactide co-glycolide (PLG) particles.
A nucleic acid construct of the present invention may also be administered by means of transformed host cells. Such cells include cells harvested from a subject. The nucleic acid vaccine construct can be introduced into such cells *in vitro* and the transformed cells can later be returned to the subject. The nucleic acid construct of the invention may integrate into nucleic acid already present in a cell by homologous recombination events. A transformed cell may, if desired, be grown up *in vitro* and one or more of the resultant cells may be used in the present invention. Cells can be provided at an appropriate site in a patient by known surgical or microsurgical techniques (e.g. grafting, micro-injection, etc.). Suitable cells include dendritic cells.

The amount of vaccine composition which is delivered will vary significantly, depending upon the species and weight of mammal being immunised, the nature of the disease state

being treated/protected against, the vaccination protocol adopted (i.e. single administration versus repeated doses), the route of administration and the potency and dose of the adjuvant compound chosen. Based upon these variables, a medical or veterinary practitioner will readily be able to determine the appropriate dosage level but it may be, for example, when the vaccine is a nucleic acid that the dose will be 0.5-5μg/kg of the nucleic acid constructs or composition containing them. In particular, the dose will vary depending on the route of administration. For example, when using intradermal administration on gold beads, the total dosage will preferably between 1μg – 10ng, particularly preferably, the total dosage will be between 10μg and 1ng. When the nucleic acid construct is administered directly, the total dosage is generally higher, for example between 50μg and 1 or more milligram. The above dosages are exemplary of the average case.

In a protein vaccine, the amount of protein in each vaccine dose is selected as an amount which induces an immunoprotective response without significant, adverse side effects in typical vaccinees. Such amount will vary depending upon which specific immunogen is employed and how it is presented. Generally, it is expected that each dose will comprise 1-1000 μg of protein, preferably 1-500 μg, preferably 1-100μg, most preferably 1 to 50μg. An optimal amount for a particular vaccine can be ascertained by standard studies involving observation of appropriate immune responses in vaccinated subjects. Following an initial vaccination, subjects may receive one or several booster immunisation adequately spaced. Such a vaccine formulation may be either a priming or boosting vaccination regime; be administered systemically, for example *via* the transdermal, subcutaneous or intramuscular routes or applied to a mucosal surface via, for example, intra nasal or oral routes.

There can, of course, be individual instances where higher or lower dosage ranges are merited, and such are within the scope of this invention.

It is possible for the vaccine composition to be administered on a once off basis or to be administered repeatedly, for example, between 1 and 7 times, preferably between 1 and 4 times, at intervals between about 1 day and about 18 months, preferably one month. This may

be optionally followed by dosing at regular intervals of between 1 and 12 months for a period up to the remainder of the patient's life. In an embodiment the patient will receive the antigen in different forms in a prime boost regime. Thus for example an antigen will be first administered as a DNA based vaccine and then subsequently administered as a protein adjuvant base formulation. Once again, however, this treatment regime will be significantly varied depending upon the size and species of animal concerned, the amount of nucleic acid vaccine and / or protein composition administered, the route of administration, the potency and dose of any adjuvant compounds used and other factors which would be apparent to a skilled veterinary or medical practitioner.

The present invention is exemplified, but not limited to, the following examples.

For the methods below the following nomenclature applies:

1. The construct called mouse IL13 (mIL-13) with p30 inserted into the protein (substituted for the looped region between alpha helices C and D of mouse IL13) is refered to as mIL13p30CD.
2. The construct called mouse IL13 with p30 at the N-terminus, is refered to as mIL13p30.
3. The construct called new chimaeric IL13 design with p30 N-terminus, is refered to as cIL13new.

IL-13 subcloning/ modifications:

mIL-13 CD cDNA was synthesized tailing 5' with *Kpn* I and 3' with *Bam* HI restriction enzyme recognition sequences. The synthesized gene was subcloned between the *Kpn* I and *Bam* HI restriction sites of pCDN which encodes DHFR (Aiyer et al, 1994). The resultant intermediate was subsequently modified by inserting an FC fusion. Site-directed insertional mutagenesis was used to precisely insert human IgG1 FC in frame with the 3' end coding sequence preceding the stop codon of IL-13 (Geisser et al 2001). This was performed in two steps 1. IgG1 FC was amplified from a cDNA template, pCDN-FC, using the following primer set, (Forward : 5'..CAACTGTTTCGCCACGGCCCC

TTCCTGGAGGTCCTGTTCGGTGGACCAGGATCCGAGCCCAAATCGGCCGAC...3' and Reverse: 5' ...CTAGGTAGTTGGTAACCGTTAACGG...3') in a PCR reaction catalyzed by KOD proof-reading polymerase (Novagen). 2. The resultant PCR product was gel purified and 250ng used as a targeting fragment in a site-directed mutagenesis reaction using the QuickChange kit (Stratagene) with 50ng mIL-13 CD-pCDN and 2.5 U PfuTurbo . The mutagenesis protocol consisted of 18 Cycles of 30s at 95°C, 30S at 55°C, and 16 minutes at 68°C . At the end of the mutagenesis protocol, the reaction was digested with 10U *Dpn* I to remove the original methylated wild-type template DNA. 1ul of the final digested reaction was used to transform 100ul Epicurian chemically competent *E. coli* cells (Stratagene). Recombinant clones were screened by restriction digestion and positive clones sequence confirmed fully across the FC region using IL-13 forward and pCDN reverse primers. The final plasmid, pCDNmIL13CDFC encodes a C-terminal FC fusion separated by a PreScission protease cleavage site for FC removal. Transcription is under control of the CMV promoter.

pCDNmIL13p30FC was constructed in exactly the same way as described above for pCDNmIL13CDFC using the same forward and reverse primers to generate the targeting fragment for site-directed insertion of the FC region into pCDNmIL13p30.
pCDNcIL13newFC was constructed using the following forward primer (5'..AACCTGTTTCGCCGCGGCCCCTTCCTGGAGGTCC TGTTCGGTGGACCAGGATCCGAGCCCAAATCGGCCGAC...3') and the same reverse primer described above to generate the targeting fragment for site-directed insertion of the FC region into pCDNcIL13new.

pCDN IL13oldFC was constructed by site-directed replacement of mIL13 CD within pCDNmIL13CDFC with chimeric IL13. Site-directed replacement was performed as described for site-directed insertion. cIL13 was PCR amplified from 6His-cIL13 using the following primers (Forward: 5' 5'...GTGTCTCTCC CTCTGACCCTTAGG...3' and Reverse: 5'...CAGTTGCTTTGTGTAGCTGAG CAG...3' to generate a targeting fragment for replacement into pCDNmIL13. This generates a precise fusion to the IL-13 signal sequence encoded at the 5' end and the PreScission-FC region encoded at the 3' end.

Generation of Stable CHO E1A clones:

Plasmids were stably expressed in a DHFR negative, E1A expressing line (CHO E1A, ACC317). Cells were resuspended at 1 x 10^7 cell/ml in cold phosphate buffered sucrose, transferred to a Gene Pulser Cuvette, and electroporated with 15ug *Not* I linearized plasmid at 400volt and 25uFd in a GenePulser (Biorad). Electroporated cells were plated in a 96 well plate at 2.5 x 10^3 viable cells per well in complete medium containing 1 X Nucleosides. After 48 hours the medium was exchanged with fresh medium lacking nucleosides. Cells were subsequently selected over 3-4 weeks in the absence of nucleosides. Positive clones were screened from the 96 well plate by monitoring FC expression from conditioned medium using an FC- electrochemiluminescence detection protocol (Yang, et al., 1994) on an Origen analyzer (IGEN). Positive cell lines were scaled to several liters in complete medium minus nucleosides. Fermentations were carried out at 34°C for 10-11 days. Conditioned medium was harvested and 0.2 uM sterile filtered in preparation for FC purification.

Purification:

Murine IL13CD/Fc was captured from CHO medium onto ProSep-A High Capacity resin (Bioprocessing Limited). The murine IL13CD/Fc was eluted from the ProSep-A resin with 0.1M Glycine pH=3.0, neutralized with 1M HEPES pH=7.6, and dialyzed against 25mM sodium phosphate 0.15M sodium chloride pH=7 (Spectra/Por® 7 membrane, MWCO:8000). Overall yield was 644mg murine IL13CD/Fc from 3.8 liter CHO medium.

References:

Aiyer, N, Baker, E, Wu, H-L, Nambi, P, Edwards, RM, Trill, JJ, Ellis, C, Bergsma, DJ. (1994): Human AT1 receptor is a single copy gene: characterization in a stable cell line. Molecular and Cellular Biochemistry 131:75-86.

Geiser, M, Cebe, R, Drewello, D, and Schmitz, R (2001): Integration of PCR Fragments at Any Specific Site within Cloning Vectors without the Use of Restriction Enzymes and DNA Ligase. Biotechniques 31: 88-92.

Yang, H, Leland, JK, Yost, D, Massey, RJ (1994): Electrochemiluminescence: A new diagnostic and research tool. Biotechnology, 12:193-194.

Translation Diagrams:

Key:
Black: IL-13 plus insertions
Red: Prescission site
Blue: Human IgG1 Fc

1. pCDNmIL13CDFC

```
KpnI
                                                  |
          AACCGTCAGATCGCCTGGAGACGCCATCGAATTCGGTACCGCCACCATGGCGCTCTGGGT
      961 ---------+---------+---------+---------+---------+---------+ 1020
b                                                   M  A  L  W  V  -

          GACTGCAGTCCTGGCTCTTGCTTGCCTTGGTGGTCTCGCCGCCCCAGGGCCGGTGCCACG
     1021 ---------+---------+---------+---------+---------+---------+ 1080
b          T  A  V  L  A  L  A  C  L  G  G  L  A  A  P  G  P  V  P  R  -

          TTCTGTGTCTCTCCCTCTGACCCTTAAGGAGCTTATTGAGGAGCTGAGCAACATCACACA
     1081 ---------+---------+---------+---------+---------+---------+ 1140
b          S  V  S  L  P  L  T  L  K  E  L  I  E  E  L  S  N  I  T  Q  -

          AGACCAGACTCCCCTGTGCAACGGCAGCATGGTATGGAGTGTGGACCTGGCCGCTGGCGG
     1141 ---------+---------+---------+---------+---------+---------+ 1200
b          D  Q  T  P  L  C  N  G  S  M  V  W  S  V  D  L  A  A  G  G  -

          GTTCTGTGTAGCCCTGGATTCCCTGACCAACATCTCCAATTGCAATGCCATCTACCGTAC
     1201 ---------+---------+---------+---------+---------+---------+ 1260
b          F  C  V  A  L  D  S  L  T  N  I  S  N  C  N  A  I  Y  R  T  -

          CCAGCGTATTTTGCATGGCCTCTGTAACCGCAAGTTTAATAATTTTACCGTTAGCTTTTG
     1261 ---------+---------+---------+---------+---------+---------+ 1320
b          Q  R  I  L  H  G  L  C  N  R  K  F  N  N  F  T  V  S  F  W  -
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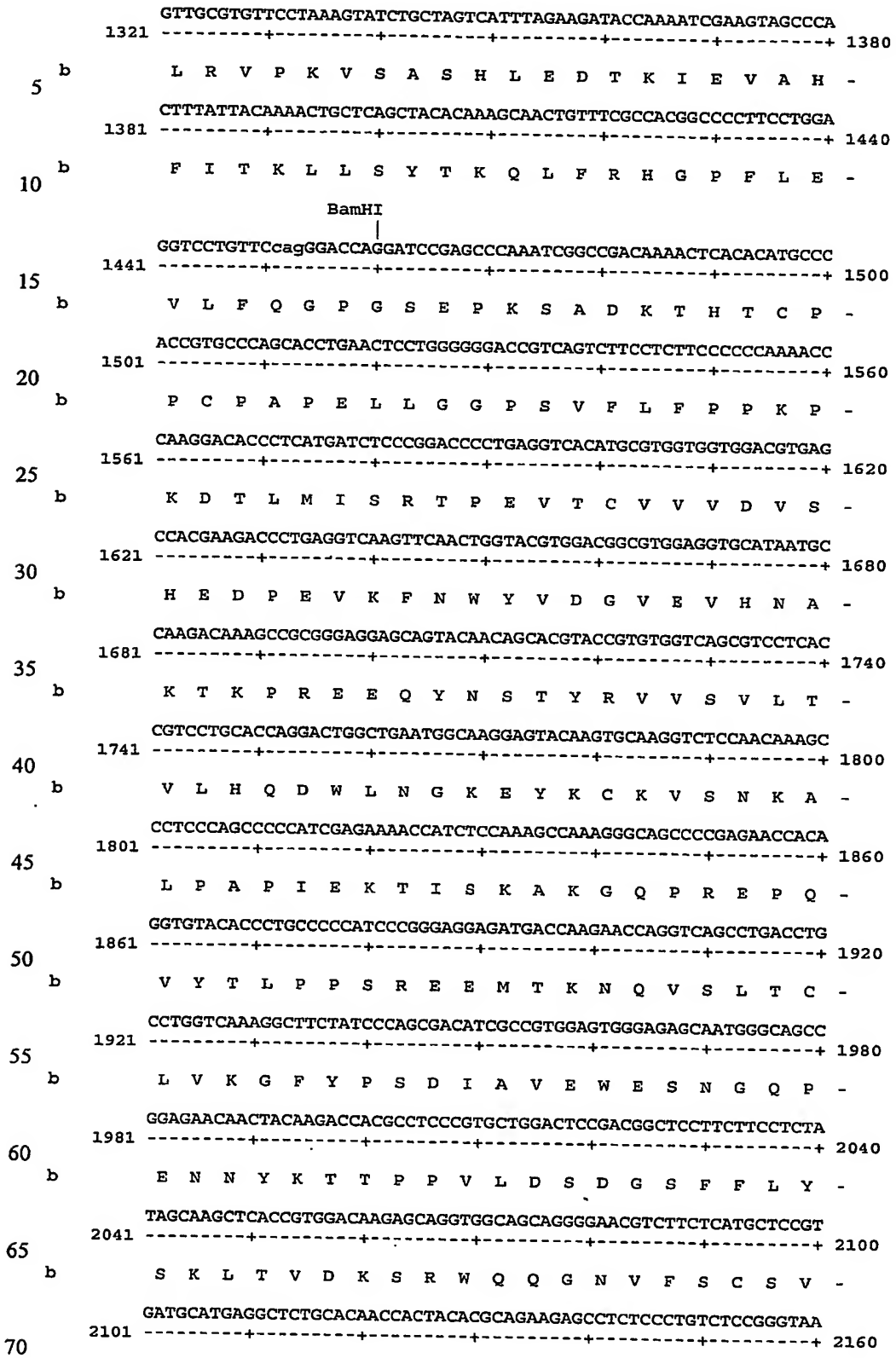
GTTGCGTGTTCCTAAAGTATCTGCTAGTCATTTAGAAGATACCAAAATCGAAGTAGCCCA
1321 ---------+---------+---------+---------+---------+---------+ 1380
b L R V P K V S A S H L E D T K I E V A H -
CTTTATTACAAAACTGCTCAGCTACACAAAGCAACTGTTTCGCCACGGCCCCTTCCTGGA
1381 ---------+---------+---------+---------+---------+---------+ 1440
b F I T K L L S Y T K Q L F R H G P F L E -
BamHI
GGTCCTGTTCcagGGACCAGGATCCGAGCCCAAATCGGCCGACAAAACTCACACATGCCC
1441 ---------+---------+---------+---------+---------+---------+ 1500
b V L F Q G P G S E P K S A D K T H T C P -
ACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACC
1501 ---------+---------+---------+---------+---------+---------+ 1560
b P C P A P E L L G G P S V F L F P P K P -
CAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAG
1561 ---------+---------+---------+---------+---------+---------+ 1620
b K D T L M I S R T P E V T C V V V D V S -
CCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGC
1621 ---------+---------+---------+---------+---------+---------+ 1680
b H E D P E V K F N W Y V D G V E V H N A -
CAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCAC
1681 ---------+---------+---------+---------+---------+---------+ 1740
b K T K P R E E Q Y N S T Y R V V S V L T -
CGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGC
1741 ---------+---------+---------+---------+---------+---------+ 1800
b V L H Q D W L N G K E Y K C K V S N K A -
CCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACA
1801 ---------+---------+---------+---------+---------+---------+ 1860
b L P A P I E K T I S K A K G Q P R E P Q -
GGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTG
1861 ---------+---------+---------+---------+---------+---------+ 1920
b V Y T L P P S R E E M T K N Q V S L T C -
CCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCC
1921 ---------+---------+---------+---------+---------+---------+ 1980
b L V K G F Y P S D I A V E W E S N G Q P -
GGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTA
1981 ---------+---------+---------+---------+---------+---------+ 2040
b E N N Y K T T P P V L D S D G S F F L Y -
TAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGT
2041 ---------+---------+---------+---------+---------+---------+ 2100
b S K L T V D K S R W Q Q G N V F S C S V -
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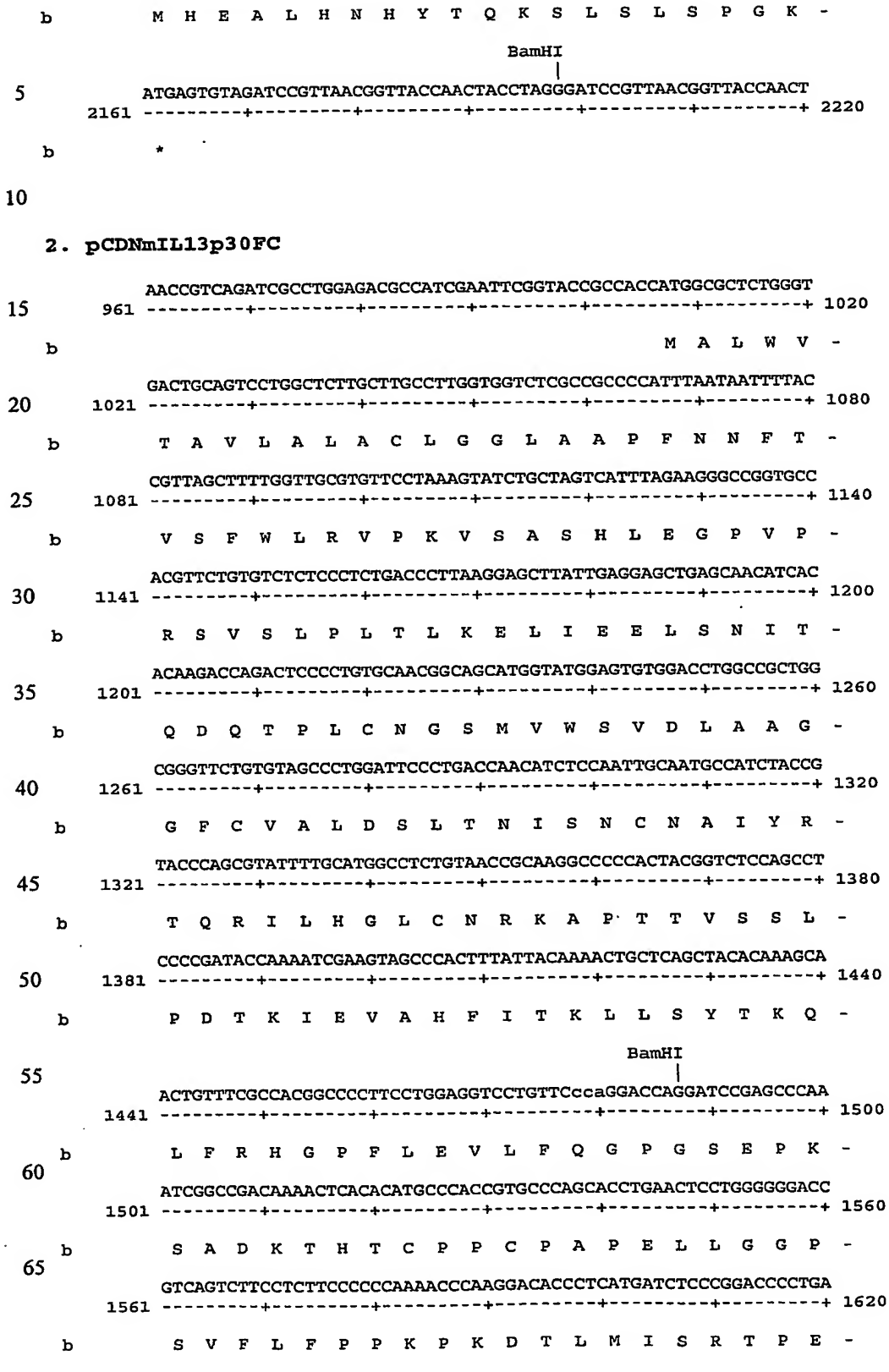
2101 ---------+---------+---------+---------+---------+---------+ 2160


b M H E A L H N H Y T Q K S L S L S P G K -
BamHI
5 ATGAGTGTAGATCCGTTAACGGTTACCAACTACCTAGGGATCCGTTAACGGTTACCAACT
2161 ---------+---------+---------+---------+---------+---------+ 2220
b *
10
2. pCDNmIL13p30FC
AACCGTCAGATCGCCTGGAGACGCCATCGAATTCGGTACCGCCACCATGGCGCTCTGGGT
15 961 ---------+---------+---------+---------+---------+---------+ 1020
b M A L W V -
GACTGCAGTCCTGGCTCTTGCTTGCCTTGGTGGTCTCGCCGCCCCATTTAATAATTTTAC
20 1021 ---------+---------+---------+---------+---------+---------+ 1080
b T A V L A L A C L G G L A A P F N N F T -
CGTTAGCTTTTGGTTGCGTGTTCCTAAAGTATCTGCTAGTCATTTAGAAGGGCCGGTGCC
25 1081 ---------+---------+---------+---------+---------+---------+ 1140
b V S F W L R V P K V S A S H L E G P V P -
ACGTTCTGTGTCTCTCCCTCTGACCCTTAAGGAGCTTATTGAGGAGCTGAGCAACATCAC
30 1141 ---------+---------+---------+---------+---------+---------+ 1200
b R S V S L P L T L K E L I E E L S N I T -
ACAAGACCAGACTCCCCTGTGCAACGGCAGCATGGTATGGAGTGTGGACCTGGCCGCTGG
35 1201 ---------+---------+---------+---------+---------+---------+ 1260
b Q D Q T P L C N G S M V W S V D L A A G -
CGGGTTCTGTGTAGCCCTGGATTCCCTGACCAACATCTCCAATTGCAATGCCATCTACCG
40 1261 ---------+---------+---------+---------+---------+---------+ 1320
b G F C V A L D S L T N I S N C N A I Y R -
TACCCAGCGTATTTTGCATGGCCTCTGTAACCGCAAGGCCCCCACTACGGTCTCCAGCCT
45 1321 ---------+---------+---------+---------+---------+---------+ 1380
b T Q R I L H G L C N R K A P T T V S S L -
CCCCGATACCAAAATCGAAGTAGCCCACTTTATTACAAAACTGCTCAGCTACACAAAGCA
50 1381 ---------+---------+---------+---------+---------+---------+ 1440
b P D T K I E V A H F I T K L L S Y T K Q -
BamHI
55
ACTGTTTCGCCACGGCCCCTTCCTGGAGGTCCTGTTCccaGGACCAGGATCCGAGCCCAA
1441 ---------+---------+---------+---------+---------+---------+ 1500
b L F R H G P F L E V L F Q G P G S E P K -
60
ATCGGCCGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACC
1501 ---------+---------+---------+---------+---------+---------+ 1560
b S A D K T H T C P P C P A P E L L G G P -
65
GTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGA
1561 ---------+---------+---------+---------+---------+---------+ 1620
b S V F L F P P K P K D T L M I S R T P E -

```
        GGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTA
     1621 ---------+---------+---------+---------+---------+---------+ 1680
 5 b       V  T  C  V  V  V  D  V  S  H  E  D  P  E  V  K  F  N  W  Y  -

        CGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAG
     1681 ---------+---------+---------+---------+---------+---------+ 1740
10 b       V  D  G  V  E  V  H  N  A  K  T  K  P  R  E  E  Q  Y  N  S  -

        CACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGA
     1741 ---------+---------+---------+---------+---------+---------+ 1800
15 b       T  Y  R  V  V  S  V  L  T  V  L  H  Q  D  W  L  N  G  K  E  -

        GTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAA
     1801 ---------+---------+---------+---------+---------+---------+ 1860
20 b       Y  K  C  K  V  S  N  K  A  L  P  A  P  I  E  K  T  I  S  K  -

        AGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGAT
     1861 ---------+---------+---------+---------+---------+---------+ 1920
25 b       A  K  G  Q  P  R  E  P  Q  V  Y  T  L  P  P  S  R  E  E  M  -

        GACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGC
     1921 ---------+---------+---------+---------+---------+---------+ 1980
30 b       T  K  N  Q  V  S  L  T  C  L  V  K  G  F  Y  P  S  D  I  A  -

        CGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCT
     1981 ---------+---------+---------+---------+---------+---------+ 2040
35 b       V  E  W  E  S  N  G  Q  P  E  N  N  Y  K  T  T  P  P  V  L  -

        GGACTCCGACGGCTCCTTCTTCCTCTATAGCAAGCTCACCGTGGACAAGAGCAGGTGGCA
     2041 ---------+---------+---------+---------+---------+---------+ 2100
40 b       D  S  D  G  S  F  F  L  Y  S  K  L  T  V  D  K  S  R  W  Q  -

        GCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCA
     2101 ---------+---------+---------+---------+---------+---------+ 2160
45 b       Q  G  N  V  F  S  C  S  V  M  H  E  A  L  H  N  H  Y  T  Q  -

        GAAGAGCCTCTCCCTGTCTCCGGGTAAATGAGTGTAGATCCGTTAACGGTTACCAACTAC
     2161 ---------+---------+---------+---------+---------+---------+ 2220
50 b       K  S  L  S  L  S  P  G  K  *  -
```

3. pCDNcIL13newFC

```
55
   KpnI
                                          |
        AACCGTCAGATCGCCTGGAGACGCCATCGAATTCGGTACCGCCACCATGGCGCTCTGGGT
      961 ---------+---------+---------+---------+---------+---------+ 1020
60
   b                                                  M  A  L  W  V  -

        GACTGCAGTCCTGGCTCTTGCTTGCCTTGGTGGTCTCGCCGCCCCATTTAATAATTTTAC
     1021 ---------+---------+---------+---------+---------+---------+ 1080
65
   b       T  A  V  L  A  L  A  C  L  G  G  L  A  A  P  F  N  N  F  T  -

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     1081 ---------+---------+---------+---------+---------+---------+ 1140
70
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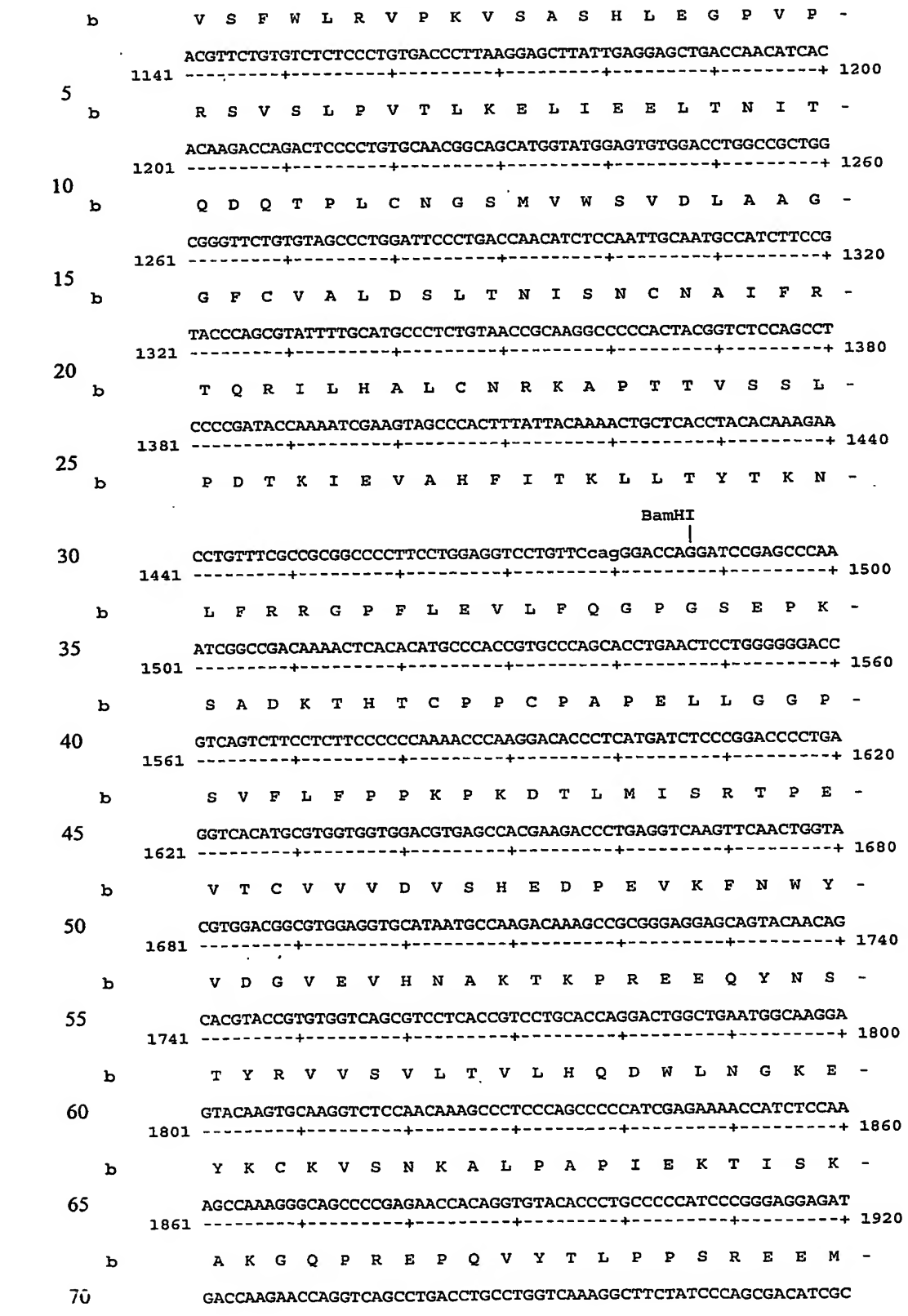
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1141 ----------+---------+---------+---------+---------+---------+ 1200
b R S V S L P V T L K E L I E E L T N I T -
ACAAGACCAGACTCCCCTGTGCAACGGCAGCATGGTATGGAGTGTGGACCTGGCCGCTGG
1201 ---------+---------+---------+---------+---------+---------+ 1260
b Q D Q T P L C N G S M V W S V D L A A G -
CGGGTTCTGTGTAGCCCTGGATTCCCTGACCAACATCTCCAATTGCAATGCCATCTTCCG
1261 ---------+---------+---------+---------+---------+---------+ 1320
b G F C V A L D S L T N I S N C N A I F R -
TACCCAGCGTATTTTGCATGCCCTCTGTAACCGCAAGGCCCCCACTACGGTCTCCAGCCT
1321 ---------+---------+---------+---------+---------+---------+ 1380
b T Q R I L H A L C N R K A P T T V S S L -
CCCCGATACCAAAATCGAAGTAGCCCACTTTATTACAAAACTGCTCACCTACACAAAGAA
1381 ---------+---------+---------+---------+---------+---------+ 1440
b P D T K I E V A H F I T K L L T Y T K N -
BamHI
CCTGTTTCGCCGCGGCCCCTTCCTGGAGGTCCTGTTCcagGGACCAGGATCCGAGCCCAA
1441 ---------+---------+---------+---------+---------+---------+ 1500
b L F R R G P F L E V L F Q G P G S E P K -
ATCGGCCGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGGACC
1501 ---------+---------+---------+---------+---------+---------+ 1560
b S A D K T H T C P P C P A P E L L G G P -
GTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGA
1561 ---------+---------+---------+---------+---------+---------+ 1620
b S V F L F P P K P K D T L M I S R T P E -
GGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTA
1621 ---------+---------+---------+---------+---------+---------+ 1680
b V T C V V V D V S H E D P E V K F N W Y -
CGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAG
1681 ---------+---------+---------+---------+---------+---------+ 1740
b V D G V E V H N A K T K P R E E Q Y N S -
CACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGA
1741 ---------+---------+---------+---------+---------+---------+ 1800
b T Y R V V S V L T V L H Q D W L N G K E -
GTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAA
1801 ---------+---------+---------+---------+---------+---------+ 1860
b Y K C K V S N K A L P A P I E K T I S K -
AGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGAT
1861 ---------+---------+---------+---------+---------+---------+ 1920
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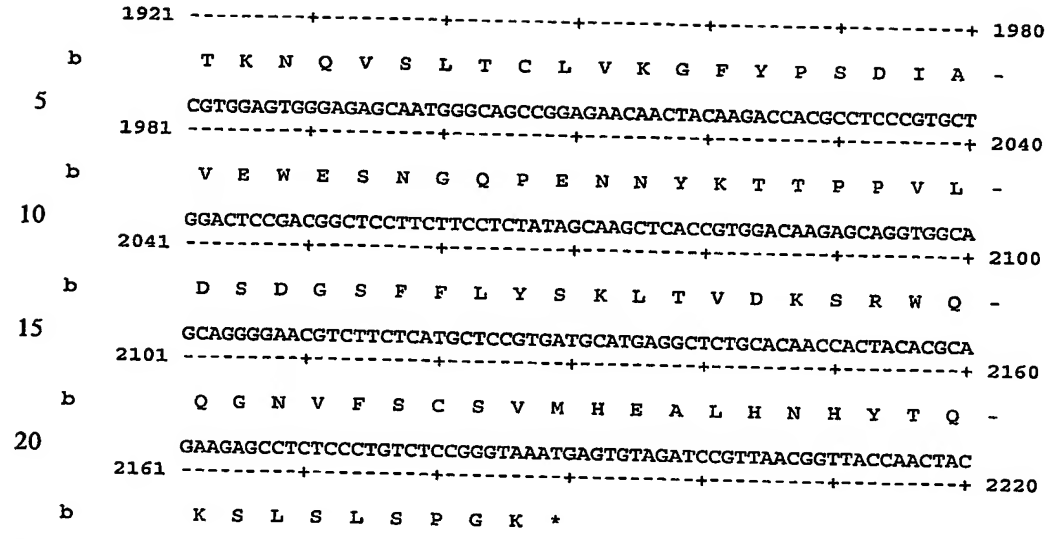
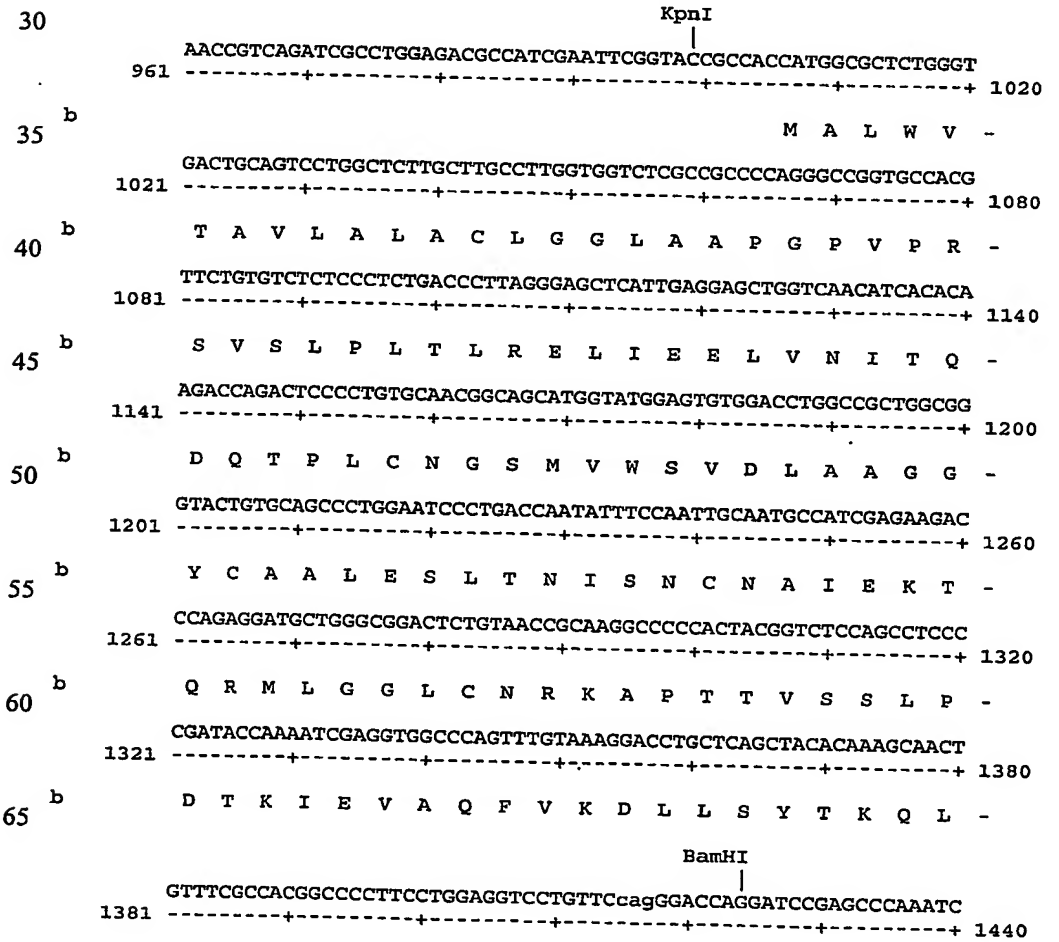
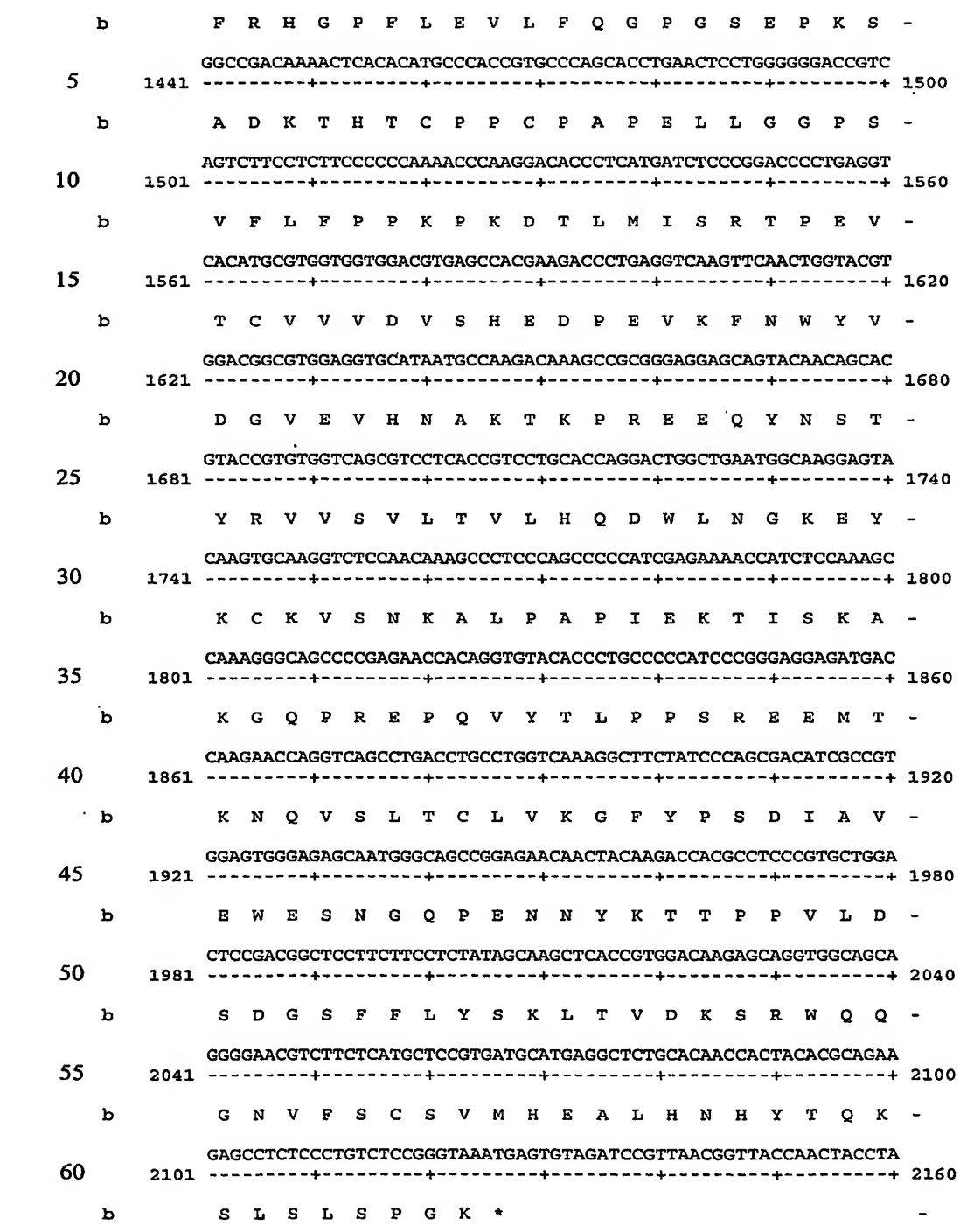


4. pCDN IL13oldFC

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b A D K T H T C P P C P A P E L L G G P S -
AGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGT
1501 ---------+---------+---------+---------+---------+---------+ 1560
b V F L F P P K P K D T L M I S R T P E V -
CACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGT
1561 ---------+---------+---------+---------+---------+---------+ 1620
b T C V V V D V S H E D P E V K F N W Y V -
GGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCAC
1621 ---------+---------+---------+---------+---------+---------+ 1680
b D G V E V H N A K T K P R E E Q Y N S T -
GTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTA
1681 ---------+---------+---------+---------+---------+---------+ 1740
b Y R V V S V L T V L H Q D W L N G K E Y -
CAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGC
1741 ---------+---------+---------+---------+---------+---------+ 1800
b K C K V S N K A L P A P I E K T I S K A -
CAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGAC
1801 ---------+---------+---------+---------+---------+---------+ 1860
b K G Q P R E P Q V Y T L P P S R E E M T -
CAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGT
1861 ---------+---------+---------+---------+---------+---------+ 1920
b K N Q V S L T C L V K G F Y P S D I A V -
GGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGA
1921 ---------+---------+---------+---------+---------+---------+ 1980
b E W E S N G Q P E N N Y K T T P P V L D -
CTCCGACGGCTCCTTCTTCCTCTATAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCA
1981 ---------+---------+---------+---------+---------+---------+ 2040
b S D G S F F L Y S K L T V D K S R W Q Q -
GGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAA
2041 ---------+---------+---------+---------+---------+---------+ 2100
b G N V F S C S V M H E A L H N H Y T Q K -
GAGCCTCTCCCTGTCTCCGGGTAAATGAGTGTAGATCCGTTAACGGTTACCAACTACCTA
2101 ---------+---------+---------+---------+---------+---------+ 2160
b S L S L S P G K * -

Claims

1. An immunogen for vaccination of a host, the immunogen comprising the autologous sequence of IL-13 for that host and foreign T-helper epitopes.
2. An immunogen as claimed in claim 1 wherein the T-helper epitopes are not derived from an orthologous IL-13 sequence.
3. An immunogen as claimed in claim 1 wherein the T-helper epitopes are P2 or P30 from tetanus toxin.
4. An immunogen as claimed in any one of claims 1 to 4 wherein the host is a human and the IL-13 sequence is a human sequence.
5. An immunogen described in SEQ ID NOs 1, 2, 3, 4, 5 or 6.
6. A method of treatment of asthma or COPD in a human comprising administering a vaccine comprising an immunogen as claimed in any one of claims 1 to 5.